

# Spatio-selection in Expanding Bacterial Colonies

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## Abstract

Segregation of populations is a key question in evolution theory. One important aspect is the relation between spatial organization and the population's composition. Here we study a specific example – sectors in expanding bacterial colonies. Such sectors are spatially segregated sub-populations of mutants. The sectors can be seen both in disk-shaped colonies and in branching colonies. We study the sectors using two models we have used in the past to study bacterial colonies – a continuous reaction-diffusion model with non-linear diffusion and a discrete “Communicating Walkers” model. We find that in expanding colonies, and especially in branching colonies, segregation processes are more likely than in a spatially static population. One such process is the establishment of stable sub-population having neutral mutation. Another example is the maintenance of wild-type population along side with sub-population of advantageous mutants. Understanding such processes in bacterial colonies is an important subject by itself, as well as a model system for similar processes in other spreading populations.

## I. INTRODUCTION

Charles Darwin's theory of evolution was inspired by his observations at the Galapagos Islands [1]. These observations of small differences between related species led him to identify

the importance of mutations and to create the theory of natural selection. The speciation he saw was intimately related to spatial structure – the geographical separation of the islands – and to temporal dynamics – the spreading of birds and animals from island to island. The geographical separation prevented the occupants of a new island from being mixed back into the population from which they came. The geographical separation soon turned into segregation of the population into genetically different groups – the first step towards speciation. Aside from natural selection, another evolutionary ”force” acting in this case is genetic drift. Genetic drift is the process where due to fluctuations in a small population a mutation can spread in the entire population even if it is selectively neutral or even slightly disadvantageous.

Recently, pattern formation in bacterial colonies became the focus of attention [2,3,4,5,6,7,8,9,10,11,12,13,14,15]. In this paper we study the subject of expression of mutations, and especially segregation of populations, in the context of expanding bacterial colonies. We take the bacterial colonies to be a model system for the study of expression of mutations in a spreading population, as well as an interesting and important subject by itself.

During the course of evolution, bacteria have developed sophisticated cooperative behavior and intricate communication capabilities [16,17,18,19,20]. These include: direct cell-cell physical interactions via extra- membrane polymers [21,22], collective production of extra-cellular ”wetting” fluid for movement on hard surfaces [3,23], long range chemical signaling, such as quorum sensing [24,25,26] and chemotactic signaling<sup>1</sup> [27,28,29], collective activation and deactivation of genes [30,31,4] and even exchange of genetic material [32,33,34]. Utilizing these capabilities, bacterial colonies develop complex spatio-temporal patterns in response to adverse growth conditions.

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<sup>1</sup>Chemotaxis is a bias of movement according to the gradient of a chemical agent. Chemotactic signaling is a chemotactic response to an agent emitted by the bacteria.

Fujikawa and Matsushita [6,35,36] reported for the first time<sup>2</sup> that bacterial colonies could grow elaborate branching patterns of the type known from the study of fractal formation in the process of diffusion-limited-aggregation (DLA) [39,40,41]. This work was done with *Bacillus subtilis*, but was subsequently extended to other bacterial species such as *Serratia marcescens* and *Salmonella anatum* [42]. It was shown explicitly that nutrient diffusion was the relevant dynamics responsible for the growth instability. Motivated by these observations, Ben-Jacob *et al.* [8,43,10] conducted new experiments to see how adaptive bacterial colonies could be in the presence of external "pressure", here in the form of a limited nutrient supply and hard surface. The work was done with a newly identified species, *Paenibacillus dendritiformis* var. *dendron* [44]. This species is motile on the hard surface and its colonies exhibit branching patterns (Fig. 1).

There is a well known observed (but rarely studied) phenomenon of bursts of new sectors of mutants during the growth of bacterial colonies (see for example Fig. 2 and Refs [30,45]). Actually, the phenomenon is more general. Fig. 3 (taken from [46]) shows an emerging sector in a yeast colony. If the mutants have the same growth dynamics as the "normal", wild-type, bacteria they will usually go unnoticed (unless some property such as coloring distinguish them)<sup>3</sup>. If, however, the mutants have different growth dynamics, a distinguished sector with a different growth pattern might indicate their presence.

In a branching colony, the geometrical structure may aid the bursting of a sector of "neutral" mutants; once a branch (or a cluster of branches) is detached from his neighboring branches (detached in the sense that bacteria cannot cross from one branch to the other), the

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<sup>2</sup>We refer to the first time that branching growth was studied as such. Observations of branching colonies occurred long ago [37,38].

<sup>3</sup>Different coloring may result from different enzymatic activity (natural coloring) or from a different response to a staining process (artificial coloring). In both cases the mutation is not neutral in the strictest sense, but it is neutral as far as the dynamics is concerned.

effective population is smaller than the colony's population. In such a "reduced" population, genetic drift is more probable and a neutral mutant may take over the population in some branches. Sectors of "neutral" mutations usually go undetected – by definition their growth dynamics is identical to that of the wild-type (original) bacteria and no geometrical feature highlights the sectors.

Sectors of advantageous mutation are much easier to detect, as they usually grow in a somewhat different pattern. An advantage in this context might be faster multiplication, higher motility or elevated sensitivity to chemotactic materials. In all those cases the mutants have an advantage in accessing food resources. In a pre-set geometry (or without spatial structure) the mutants might starve the wild-type bacteria and drive them to extinction. But in a spreading colony each part of the colony is heading in a different direction, thus the two populations can co-exist. The dynamic process of spreading aids the segregation of the population.

The first analytical study of spatial spread of mutations was done by Fisher [47]. He studied the spread of advantageous mutation in the limit of large, spatially uniform population, using the Fisher-Kolmogorove equation. This equation describes the time evolution of a field representing the fraction of the mutants in the local population. The same equation can be taken to describe the spreading of a population into an uninhabited space, in which case the field represents the density of the bacteria. To study mutants by this description one must extend the model to include two fields standing for two different types of bacteria. Since these equations are expressed in the continuous limit, it excludes a-priori the effect of genetic drift. As we discuss elsewhere [48], the Fisher equation has other shortcomings that make it unsuitable for modeling bacterial colonies.

To study the sectors in the bacterial colonies we use generic models, i.e. models that adhere as much as possible to biological data, but only to details which are needed to understand the subject. The generic models can be grouped into two main categories: 1. Continuous or reaction-diffusion models [49,50]. In these models the bacteria are represented by their 2D density, and a reaction-diffusion equation of this density describes their time

evolution. This equation is coupled to the other reaction-diffusion equations for fields of chemicals, such as nutrient. 2. Discrete models such as the Communicating Walkers model of Ben- Jacob *et al.* [10,51,13] and the Bions model of Kessler and Levine [52,53]. In this approach, the microorganisms (bacteria in the first model and amoebae in second) are represented by discrete entities (walkers and bions, respectively) which can consume nutrient, reproduce, perform random or biased movement, and produce or respond to chemicals. The time evolution of the nutrient and the chemicals is described by reaction-diffusion equations. In the context of branching growth of bacterial colonies, the continuous modeling approach has been pursued recently by Mimura and Matsushita *et al.* [54,55], Kawasaki *et al.* [56] and Kitsunezaki [57]. In [48] we present a summary and critique of this approach (also see [58]).

In the current study, we use both discrete and continuous models, altered to include two bacterial types. In some cases (but not all) the two bacterial types have different growth dynamics. We begin each run with a uniform population. The event of mutation is included with some finite probability of the wild-type strain changing into a mutant during the process of multiplication.

Representing mutations in the above two modeling schemes gives rise to possible problems. In a continuous model there is a problem representing a single mutation because the equations deal with bacterial area density, not with individual bacterium. In a previous paper we have studied [59] the inclusion of finite size effects in the continuous model via a cutoff in the dynamics. For the study of mutations, we use as our basic "mutation unit" the cutoff density (see below). The value of this density is in the order of a single bacterium in an area determined by the relevant diffusion length (the idea of using a cutoff density to represent discrete entities was first raised by Kessler and Levine [60]). In the discrete model, each "walker" represents not one bacterium, but many bacteria ( $10^4 - 10^6$ ) [10]. Thus, a mutation of one walker means the collective mutation of all the bacteria it represents.

Note that in this paper we do not discuss the origin of the mutations. The common view in biology is that all mutations are random. Both Darwin's original view [61] and modern experiments in microbiology [62,63,64,65] suggest the possibility of mutations designed by

the bacteria as a response to a specific stressful condition. Since the stress in this case cannot be accurately assessed by a single bacterium, another possibility is that the colony as a whole designs the mutation in response to the environmental conditions. It is not necessary that only the descendants of a single cell will have the mutation; the bacteria have the means [32,33,34] to perform "horizontal inheritance" i.e. to transfer genetic information from cell to cell. If such autocatalytic or cooperative mutation occurs in the experiments, then a mutating walker in the Communicating Walkers model might be an accurate model after all.

In the next section we present the experimental observations of bacterial colonies, mutations and sectors in bacterial colonies. In section III we present the two models used in this study. Section IV presents the study itself; the simulated colonies, the results, and comparison with the experimental observation. We conclude (section V) with a short discussion of the results and possible implications to other issues, such as growth of tumors and diversification of populations.

## II. OBSERVATIONS OF COLONIAL DEVELOPMENT

In this section, we focus on the phenomena observed during the growth of *P. dendritiformis* var. *dendron*.

### A. Growth Features

As was mentioned, the typical growth pattern on semi-solid agar is a branching pattern, as shown in Fig. 1. The structure of the branching pattern varies for different growth conditions, as is demonstrated in Fig. 4.

Under the microscope, bacterial cells are seen to perform a random-walk-like movement in a layer of fluid on the agar surface (Fig. 5). This wetting fluid is assumed to be excreted by the cells and/or drawn by the cells from the agar [10,66]. The cellular movement is confined to this fluid; isolated cells spotted on the agar surface do not move. The fluid's

boundary thus defines a local boundary for the branch. Whenever the cells are active, the boundary propagates slowly, as a result of the cellular movement and production of wetting fluid.

The various colonial patterns can be grouped into several “essential patterns” or morphologies [10,43]. In order to explain the various growth morphologies, we have suggested that bacteria use *chemotactic signaling* when confronted with adverse growth conditions [10,67,68]. Chemotaxis means changes in the movement of the cell in response to a gradient of certain chemical fields [69,70,71,72]. The movement is biased along the gradient either in the gradient direction (attractive chemotaxis towards, for example, food) or in the opposite direction (repulsive chemotaxis away from, for example, oxidative toxins). Usually chemotactic response means a response to an externally produced field as in the case of chemotaxis towards food. However, the chemotactic response can be also to a field produced directly or indirectly by the bacterial cells. We will refer to this case as chemotactic signaling.

At very low agar concentrations, 0.5% and below, the colonies exhibit compact patterns, as shown in Fig. 6. Microscopic observations reveal that in this case, the bacteria swim within the agar. Thus, there is no “envelope” to the colony, and hence no branching pattern emerges (see [48,73]).

## B. Observations of Sectors

The bursting of sectors can be observed both during compact and branching growth. Examples of the first kind are shown in Figs. 7, while examples of the latter are shown in Figs. 8,9. As we can see from the pictures, sectors emerging during branching growth have a greater variety of structure and shapes than those emerging from compact colonies.. This is demonstrated by Fig. 8 depicting colonies at intermediate levels of nutrients and agar, and Fig. 9, showing colonies grown at high nutrient level and at the presence of antibiotics. Note that the sector on the left side of Fig. 9 is much more expanded than that on the right,

probably because it has irrupted at an earlier stage of the colonial development <sup>4</sup>.

Throughout this paper, we use the term “mutant” to describe the population in the emerging sectors. We have not verified the existence of a genetic difference between the bacteria in the sector and those in the rest of the colony. We have, however, verified that the phenotypic difference between the two populations is inheritable, using inoculation.

Below we shall see that it is sometimes possible to relate the shape of the sector, and the way it “bursts” out of the colony, with the specific kind of advantage that the mutants possess over the original bacteria.

### III. MODELS

We now describe the two models we have used to study the development of a colony consisting of two bacterial strains. Both models are based on the ones originally used to study a single strain colony [48,19,59,10].

#### A. The Continuous Model

A number of continuous models have been proposed to describe colonial development [60,57,55,56]. Following [48], the model we take includes a linear growth term and a non-linear diffusion of the bacteria (representing the effect of a lubricating fluid [73]). In the case of a single strain, the time evolution of the 2D bacterial density  $b(\mathbf{x}, t)$  is given by:

$$\frac{\partial b}{\partial t} = \nabla(D(b)\nabla b) + \varepsilon n b \Theta(b - \beta) - \mu b \quad ((1))$$

The first term on the RHS describes the bacterial movement, with  $D(b) = D_0 b^k$  ( $D_0$  and  $k > 0$  constants) [57]. The second term is the population growth, which is proportional to food consumption.  $n(\mathbf{x}, t)$  is the nutrient 2D density and  $\varepsilon$  the nutrient→bacteria conversion

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<sup>4</sup>Such an early irrupted sector might indicate a mixed population in the initial inoculum, and not a new mutant.



factor. The growth term is multiplied by a step function  $\Theta(b - \beta)$ , which sets it to zero if the bacterial density is smaller than a threshold  $\beta$ . This threshold represents the discreteness of the bacteria [60]. We have previously shown that the effect of the step function is negligible for small  $\beta$  [59], but we also use it when implementing the modeling of mutations. The third term describes bacterial transformation into stationary, pre-spore state, with  $\mu$  the sporulation rate.

In this model, the time development equations for the nutrient concentration  $n(\mathbf{x}, t)$  and the stationary bacteria concentration  $s(\mathbf{x}, t)$  are given by:

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \varepsilon b n \Theta(b - \beta) \quad ((2))$$

$$\frac{\partial s}{\partial t} = \mu b \quad ((3))$$

We include the effect of chemotaxis in the model using a *chemotactic flux*  $\vec{J}_{chem}$ , which is written (for the case of a chemorepellant) as:

$$\vec{J}_{chem} = \zeta(b) \chi(r) \nabla r \quad ((4))$$

where  $r(\mathbf{x}, t)$  is the concentration of the chemorepellant agent,  $\zeta(b) = b \cdot b^k = b^{k+1}$  is the bacterial response to the chemotactic agent [48], and  $\chi(r)$  is the chemotactic sensitivity to the repellent, which is negative for a chemorepellant. In the case of a chemoattractant, e.g. a nutrient, the expression for the flux will have an opposite sign. In the case of the “receptor law”, the sensitivity  $\chi(r)$  takes the form [74]:

$$\chi(r) = \frac{\chi_0 K}{(K + r)^2} \quad ((5))$$

with  $K$  and  $\chi_0$  constants.

The equation for  $r(\mathbf{x}, t)$  is:

$$\frac{\partial r}{\partial t} = D_r \nabla^2 r + \Gamma_r s - \Omega_r b r - \lambda_r r \quad ((6))$$

where  $D_r$  is the diffusion coefficient of the chemorepellant agent,  $\Gamma_r$  is the emission rate of repellent by the pre-spore cells,  $\Omega_r$  is the decomposition rate of the repellent by active bacteria, and  $\lambda_r$  is the rate of spontaneous decomposition of the repellent.

In order to generalize this model to study mutants, we must introduce two fields, for the densities of the wild-type bacteria (“type 1”) and the mutants (“type 2”), and allow some probability of transition from wild-type to mutants. In the absence of chemotaxis, the equations for the bacterial density of the two strains will be written (with subscript denoting bacteria type):

$$\frac{\partial b_1}{\partial t} = \nabla(D_1(b)\nabla b_1) + \varepsilon_1 n b_1 \Theta(b - \beta) - \mu_1 b_1 - F_{12} \quad ((7))$$

$$\frac{\partial b_2}{\partial t} = \nabla(D_2(b)\nabla b_2) + \varepsilon_2 n b_2 \Theta(b - \beta) - \mu_2 b_2 + F_{12} \quad ((8))$$

where  $D_{0,2} = D_{0,1} b^k$  ( $b = b_1 + b_2$ ).

Note that the mutant strain  $b_2$  includes a “source” term  $F_{12}$ , which is the rate of transition  $b_1 \rightarrow b_2$ , and is given by the growth rate of  $b_1$  multiplied by a constant mutation rate (For simplicity, we do not include the process of reverse mutations  $F_{21}$ ).

## B. The Communicating Walkers Model

The Communicating Walkers model [10] is a hybridization of the “continuous” and “atomistic” approaches used in the study of non-living systems. The diffusion of the chemicals is handled by solving a continuous diffusion equation (including sources and sinks) on a tridiagonal lattice. The bacterial cells are represented by walkers allowing a more detailed description. In a typical experiment there are  $10^9 - 10^{10}$  cells in a petri-dish at the end of the growth. Hence it is impractical to incorporate into the model each and every cell. Instead, each of the walkers represents about  $10^4 - 10^5$  cells so that we work with  $10^4 - 10^6$  walkers in one numerical “experiment”.

The walkers perform an off-lattice random walk on a plane within an envelope representing the boundary of the wetting fluid. This envelope is defined on the same triangular lattice where the diffusion equations are solved. To incorporate the swimming of the bacteria into the model, at each time step each of the active walkers (motile and metabolizing, as described below) moves a step of size  $d$  at a random angle  $\Theta$ . If this new position is

outside the envelope, the walker does not move. A counter on the segment of the envelope which would have been crossed by the movement is increased by one. When the segment counter reaches a specified number of hits  $N_c$ , the envelope propagates one lattice step and an additional lattice cell is added to the colony. This requirement of  $N_c$  hits represent the colony propagation through wetting of unoccupied areas by the bacteria. Note that  $N_c$  is related to the agar dryness, as more wetting fluid must be produced (more “collisions” are needed) to push the envelope on a harder substrate.

Motivated by the presence of a maximal growth rate of the bacteria even for optimal conditions, each walker in the model consumes food at a constant rate  $\Omega_c$  if sufficient food is available. We represent the metabolic state of the  $i$ -th walker by an ‘internal energy’  $E_i$ . The rate of change of the internal energy is given by

$$\frac{dE_i}{dt} = \kappa C_{consumed} - \frac{E_m}{\tau_R} , \quad ((9))$$

where  $\kappa$  is a conversion factor from food to internal energy ( $\kappa \cong 5 \cdot 10^3 \text{ cal/g}$ ) and  $E_m$  represent the total energy loss for all processes over the reproduction time  $\tau_R$ , excluding energy loss for cell division.  $C_{consumed}$  is  $C_{consumed} \equiv \min(\Omega_C, \Omega'_C)$  , where  $\Omega'_C$  is the maximal rate of food consumption as limited by the locally available food. When sufficient food is available,  $E_i$  increases until it reaches a threshold energy. Upon reaching this threshold, the walker divides into two. When a walker is starved for long interval of time,  $E_i$  drops to zero and the walker “freezes”. This “freezing” represents entering a pre-spore state.

We represent the diffusion of nutrients by solving the diffusion equation for a single agent whose concentration is denoted by  $n(\vec{r}, t)$ :

$$\frac{\partial n}{\partial t} = D_n \nabla^2 C - b C_{consumed} , \quad ((10))$$

where the last term includes the consumption of food by the walkers ( $b$  is their density). The equation is solved on the tridiagonal lattice. The simulations are started with inoculum of walkers at the center and a uniform distribution of the nutrient.

When modeling chemotaxis performed by walkers, it is possible to modulate the periods between tumbling (without changing the speed) in the same way the bacteria do. It can

be shown that step length modulation has the same mean effect as keeping the step length constant and biasing the direction of the steps (higher probability to move in the preferred direction). As this later approach is numerically simpler, this is the one implemented in the Communicating Walkers model. As in the aforementioned continuous model, an additional equation is written for the time evolution of the chemorepellant.

When dealing with two bacterial strains, each walker in the simulation belongs to either “type 1” (wild-type) or “type 2” (mutant), which may differ in their various biological parameters, such as step length (i.e. motility) or sensitivity to chemotaxis. The colony is initialized with an inoculation of wild-type walkers, which – when multiplying – have some finite probability of giving birth to a mutant. The two populations then co-evolve according to the dynamics described above.

## IV. RESULTS

### A. Compact growth

We start by examining mutations in colonies grown on soft agar, where growth is compact. We expect that in this case a neutral mutation will not form a segregated sector. The mutant does, however, increase its relative part of the total population in a sector of the colony (figure 10). In other words, due to the expansion of the colony, an initially tiny number of mutants gradually becomes a significant part of the total population in a specific area. Experimentally, if the mutant has some distinguishable feature – e.g. color – a sector will be observed.

Next we study the more interesting case of superior mutants. In this case one observes a sector which grows faster than the rest of the colony (see figure 7). In the simulations, a sharp segregation is obtained when the mutant is endowed with a higher growth rate  $\varepsilon$  (figure 11) or a higher motility (larger  $D_0$ , figure 12). Figure 13 depicts the results obtained by simulations of the Communicating Walker model (with the mutant having a larger step

length, which is equivalent to a higher diffusion coefficient). As can be seen, both models exhibit a fan-like sector of mutants, very similar to the one observed in the experiments. The “mixing area”, where both strains are present, is narrow, and its width is related to the width of the propagating front of the colony (the area where most bacteria are alive,  $b > s$ ).

## B. Branching growth

We now turn to the case of sectoring during branching growth. As seen in figure 14, in this case there is a slow process of segregation even for a neutral mutation. This results from the fact that a particular branch may stem from a small number of bacteria, thus allowing an initially insignificant number of mutants quickly to become the majority in some branches, and therefore in some area of the colony (genetic drift in small populations).

Mutants superior in motility (figure 15) or growth rate (figure 16) form segregated fan-like sectors which burst out of relatively slow advancing colony.

## C. The effect of chemotaxis

As we have mentioned earlier, an additional important feature of the bacterial movement is chemotaxis. We start by considering neutral mutations in the case of a colony which employs repulsive chemotactic signaling. As seen in figure 17, the chemotactic response enhances the segregation of neutral mutants. This results from branches being thinner in the presence of repulsive chemotaxis, and the reduced mixing of bacteria because of the directed motion.

In the case of mutants with superior motility (figure 18), a segregated sector is formed which is not fan-like (as opposed to the case without chemotaxis), probably because of the biased, radially-oriented motion of the bacteria, coming from the long range repulsive chemotaxis.

A fan-like sector *does* appear when the mutant has a higher sensitivity to the chemotactic signals (figure 19). In this case, however, the sector is composed of a mixture of the “wild-

type” and the mutants. Figure 20 displays the result of Communicating Walker simulation for this case<sup>5</sup>. Note the similarity of both models’ results with experimental observations (figure 8).

The influence of food chemotaxis on the sectors (figures 21,22,23) is similar to that of repulsive chemotaxis.

Thus, the shape of the emerging sector (e.g. fan-like or not), and the difference between the branches in the original colony and in the sector, might testify to the nature of the advantage possessed by the mutant.

## V. DISCUSSION

In this paper we presented our study of the appearance of segregated sectors of mutants in expanding bacterial colonies. After reviewing the experimental observations of this phenomenon, we showed the results of simulations performed using two different models, the discrete “Communicating Walker” model, and a continuous reaction-diffusion model. Using these models as an aid to analytical reasoning, we are able to understand what factors – geometrical, regulatory and others – favor the segregation of the mutant population. These factors include:

1. Expansion of the colony – in the form of a finite front propagating away from areas of depleted nutrient, and towards areas of high nutrient concentration.
2. Branching patterns, where the population in each branch is much smaller than the colony’s population, making a genetic drift more probable, so that a mutant take over the whole population in a sector of the colony.
3. Chemotaxis: Food chemotaxis and repulsive chemotactic signaling cause the bacterial motion to become less random and more directed (outward and towards nutrients),

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<sup>5</sup>The discrete model shows a higher tendency for segregation here.

thus lowering mixing of populations.

4. An advantageous mutant, having e.g. a higher motility or a faster reproduction rate, will probably conquer a sector of its own and quickly become segregated. This sector will usually be fan-like, bursting out of the colony, owing to the faster expansion of the mutants, as compared to that of the wild-type population.

As in every modeling endeavour, one must note the model’s limitations along with its success in reproducing and predicting biological phenomena. As mentioned above, care must be taken when modeling discrete entities (i.e. bacteria) using a continuous model (for more on that, see [60,48,59]). This point gains even more importance when we deal with the process of mutation, which is a “single bacteria event”. The discrete Walker model is not free of this shortcoming as well, because in this model each walker represents not one bacterium, but many [10].

The observed segregation of mutant population raises some interesting evolutionary questions. Faster movement (for example) is an advantage for the bacteria, as the burst of sectors show. Why then does this mutation not take over the general population and becomes the wild-type? In other words, why were there any wild-type bacteria for us to isolate in the first place? One possible answer is that this advantage might turn out to be a *disadvantage* at different environmental conditions (e.g. inability to remain confined to some small toxin-free oasis). Another possibility is that this mutant, though possessing some superior biological feature, is lacking in another feature, essential to its long-term survival (e.g. wasting too much energy on movement when it is not advantageous).

Beyond the study of sectoring in bacterial colonies, intuition about the basic mechanisms of spatial segregation of populations might be useful for other problems. Such problems may include the important issues of growth of tumors and the diversification of populations on a macroscopic scale. Both may employ similar geometrical features and communication capabilities, leading to segregation.

Notwithstanding the above mentioned reservations, we believe this study demonstrates once more the capability of generic models to serve as a theoretical research tool, not only to study the basic patterns created by bacterial colonies, but also to gain deeper understanding of more general phenomena, such as the segregation of mutants in an expanding colony.

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## FIGURES

FIG. 1. Typical example of branching growth of the *P. dendritiformis* var. *dendron* for 1g/l peptone level and 1.5% agar concentration.

FIG. 2. Emerging sector in a *E. Coli* colony. Picture by James A. Shapiro, From “Bacteria as Multicellular Organisms” edited by J. Shapiro and M. Dworkin [75]. (C) Copyright 1997 by Oxford University Press, Inc. Used by permission of Oxford University Press.

FIG. 3. Emerging sector in a colony of *Yarrowia lipolytica*. Taken from [46], used with permission.

FIG. 4. Examples of typical patterns of *P. dendritiformis* var. *dendron* for intermediate agar concentration. (top left) At very high peptone level (peptone 12g/l, agar concentration 1.75%) the pattern is compact. (top right) At high peptone level (3g/l, agar 2%) the pattern is of dense fingers with pronounced radial symmetry – similar to patterns observed in Hele-Shaw cell. (bottom left) At intermediate peptone level (1g/l, agar 1.75%) the pattern is ”bushy” fractal-like pattern, with branch width smaller than the distance between branches. (bottom right) At low peptone level (0.1g/l, agar 1.75%) there are fine radial branches with apparent circular envelope.

FIG. 5. A closer look on branches of a colony: (left) Numarsky (polarized light) microscopy shows the height of the branches and their envelope. What is actually seen is the layer of lubrication fluid, not the bacteria. (right) X50 magnification shows the bacteria inside a branch. Each bar is a single bacterium. There are no bacteria outside the branch.

FIG. 6. A compact growth pattern of *P. dendritiformis* var. *dendron*, obtained when the agar surface is very soft (0.4% agar concentration and 0.1 g/l peptone).

FIG. 7. Emerging sectors in compact colonies of *P. dendritiformis*. (left) var. *dendron*, 10 g/l peptone, 0.5% agar. (middle) var. *chiralis*, 1.5 g/l peptone, 0.4% agar. (right) var. *chiralis*, 10 g/l peptone, 0.4% agar.



FIG. 8. Emerging sectors in branching colonies of *P. dendritiformis* var. *dendron*, obtained at 1 g/l peptone and 1.75% agar (left), 0.8 g/l peptone and 2% agar (right).

FIG. 9. Emerging sectors in branching colonies of *P. dendritiformis* var. *dendron*, obtained at 5 g/l peptone and 1.75% agar, in the presence of the antibiotics Stromocine.

FIG. 10. Neutral mutation in a compact colony: Results of simulation of the continuous model. It is seen that the wildtype covers the complete colony area uniformly (left). However, the mutants-to-wildtype ratio increases in a sector of the colony (right), indicating the tendency toward segregation.

FIG. 11. Mutant with a higher growth rate ( $\varepsilon_2 > \varepsilon_1$  in the continuous model), compact colony. (left) The mutant (light) irrupts in a fan-like sector from the wildtype (dark) colony. (right) The wildtype does not penetrate the mutation sector.

FIG. 12. Mutant with a higher motility ( $D_{02} > D_{01}$  in the continuous model), compact colony. (left) The mutant (light) irrupts in a slice-like sector from the wildtype (dark) colony. (right) The wildtype does not penetrate the mutation sector (right).

FIG. 13. Mutant with a higher motility (larger step length in the Communicating Walker model), compact colony.

FIG. 14. Neutral mutation in a branching colony: Results of simulation of the continuous model. It is seen that the mutant gradually becomes a majority of the population in a sector of the colony (right), while the wildtype is gradually “expelled” from this area (left).

FIG. 15. Mutant with a higher motility ( $D_{02} > D_{01}$  in the continuous model), branching colony. (left) The mutant (light) irrupts in a sector from the wildtype (dark) colony. (right) The wildtype does not penetrate the mutation sector.

FIG. 16. Mutant with a higher growth rate ( $\varepsilon_2 > \varepsilon_1$  in the continuous model), branching colony. (left) The mutant (light) irrupts in a fan-like sector from the wildtype (dark) colony. (right) The wildtype does not penetrate the mutation sector.

FIG. 17. Neutral mutation in a branching colony, with the presence of repulsive chemotactic signaling: Results of simulation of the continuous model. It is seen that the mutant gradually becomes a majority of the population in a sector of the colony (right), while the wildtype is gradually “expelled” from this area (left).

FIG. 18. Mutant with a higher motility ( $D_{0_2} > D_{0_1}$  in the continuous model), branching colony with presence of repulsive chemotactic signaling. The mutant (light) irrupts in a sector from the wildtype (dark) colony (left). Note that the sector does not burst out of the rest of the colony. (right) The wildtype does not penetrate the mutation sector.

FIG. 19. Mutant with a higher sensitivity to repulsive chemotactic signaling ( $\chi_{0_2} > \chi_{0_1}$  in the continuous model), branching colony. The mutant irrupts in a fan-like sector from the colony of wildtype bacteria (left). However, the sector is not a segregated area, and contains wildtype bacteria as well (right).

FIG. 20. Mutant with a higher sensitivity to repulsive chemotactic signaling: Results of the Communicating Walker model, branching colony. as in the continuous model, the mutant irrupts in a fan-like sector from the colony of wildtype bacteria.

FIG. 21. Neutral mutation in a branching colony, with the presence of food chemotaxis: Results of simulation of the continuous model. It is seen that the mutant gradually becomes a majority of the population in a sector of the colony (right), while the wildtype is gradually “expelled” from this area (left).

FIG. 22. Mutant with a higher sensitivity to food chemotaxis ( $\chi_{0_2} > \chi_{0_1}$  in the continuous model), branching colony. (left) The mutant (light) irrupts in a fan-like sector from the wildtype (dark) colony. (right) The wildtype does not penetrate the mutation sector.

FIG. 23. Mutant with a higher motility ( $D_{0_2} > D_{0_1}$  in the continuous model), branching colony with presence of food chemotaxis. (left) The mutant (light) irrupts in a sector from the wildtype (dark) colony. Note that the sector does not burst out of the rest of the colony. (right) The wildtype does not penetrate the mutation sector.

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